R. Bras. Zootec., 51:e20220077, 2022
https://doi.org/10.37496/rbz5120220077

Non-ruminants
Short communication

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Capsaicinoids affect intestinal mRNA expression of genes related to oxidative stress in broilers

Bruna Strieder Kreuz[1], Marcio de Souza Duarte[2], Luiz Fernando Teixeira Albino[1], Samuel Oliveira Borges[1], Maria Clara Neres Piazza[1], Marcela Eduarda Silva de Carvalho[1], João Victor de Souza Miranda[1], Arele Arlindo Calderano[1,3]*

[1] Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG, Brasil.
[2] University of Guelph, Department of Animal Biosciences, Guelph, ON, Canada.
[3] Universidade Federal de Viçosa, Laboratório Multiusuário de Biologia Muscular e Nutrigenômica, Viçosa, MG, Brasil.

ABSTRACT - This study was conducted to evaluate the effects of dietary supplementation with capsaicinoids on the performance and gene expression of broilers. At 18 days of age, 120 male broilers chickens (Cobb 500) were distributed in a completely randomized design with three treatments, eight replicates, and five birds per experimental unit. The treatments were a basal diet or basal diet with the addition of 1 or 2 mg of capsaicinoids/kg of diet. The birds had free access to water and feed throughout the experimental period (18 to 26 days of age). Broiler performance was evaluated at 26 days of age, and one bird per experimental unit was selected to collect serum and jejunum samples. Jejunum samples were used to analyze the mRNA content. Data were analyzed using one-way ANOVA, and the means were compared with Tukey's test at a significance of 0.05. There was no effect of capsaicinoid supplementation on performance, serum metabolites, or the expression of glutathione peroxidase mRNA in the jejunum of broilers. However, broilers supplemented with capsaicinoids showed a higher mRNA expression of Cu, Zn-superoxide dismutase and a reduced mRNA expression of nuclear factor-κB in the jejunum. Supplementation with 1 and 2 mg/kg capsaicinoids did not improve the performance of broilers from 18 to 26 days of age but increased the mRNA expression of Cu, Zn-superoxide dismutase and reduced the mRNA expression of nuclear factor-κB in the jejunum of broilers.

Keywords: antioxidant, capsaicin, poultry

***Corresponding author:**
calderano@ufv.br

Received: May 20, 2022
Accepted: September 26, 2022

How to cite: Kreuz, B. S.; Duarte, M. S.; Albino, L. F. T.; Borges, S. O.; Piazza, M. C. N.; Carvalho, M. E. S.; Miranda, J. V. S. and Calderano, A. A. 2022. Capsaicinoids affect intestinal mRNA expression of genes related to oxidative stress in broilers. Revista Brasileira de Zootecnia 51:e20220077. https://doi.org/10.37496/rbz5120220077

1. Introduction

Reactive oxygen species (ROS) are normally produced in mitochondria during aerobic cellular metabolism. However, oxidative stress in cells/tissues results from an imbalance between free radical production and endogenous antioxidant defense and leads to lipid peroxidation, protein nitration, DNA damage, and apoptosis (Mishra and Jha, 2019).

Oxidative stress homeostasis is one of the pillars of gut health maintenance and good productive performance of broilers (Chalvon-Demersay et al., 2021). However, in farming conditions, broilers are exposed to several environmental, technological, chemical, and nutritional stressors, resulting in potential oxidative stress and dysfunction of gut barrier and transport function (Patra, 2020), thus

limiting their productive potential (Bacou et al., 2021). Factors such as thermal stress, mycotoxins, and lipopolysaccharide exposure can induce excessive activation of nuclear factor-κB (NF-κB) and lead to detrimental consequences, including chronic inflammation, compromised health status, and decreased productive performance (Surai et al., 2021).

Natural plant bioactives and antioxidants can be important alternatives to improve poultry production and gut health, including antioxidant stress and transport function (Patra, 2020; Xue et al., 2020). Capsaicinoids are secondary metabolites responsible for the strong and hot taste of pepper fruits that are known for their pungency (Hernández-Pérez et al., 2020). Capsaicin and dehydrocapsaicin are the predominant molecules, representing approximately 90% of the total capsaicinoids, and other compounds, such as nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin, are also present in minor amounts (Giuffrida et al., 2013). Previous studies have indicated that supplementation with a combination of plant essential oils, including capsaicinoids, improves the intestinal health, antioxidative status, and performance of broilers (Karadas et al., 2014; Pirgozliev et al., 2019; Xue et al., 2020). However, studies are needed to investigate the effects of isolated capsaicinoid supplementation.

In this study, we hypothesized that capsaicinoids have antioxidant effects and that their dietary supplementation can improve broiler performance. Therefore, we aimed to evaluate the effects of dietary supplementation with a source of capsaicinoids on the performance and gene expression of broilers.

2. Material and Methods

2.1. Ethical matters

The Institutional Animal Care and Use Committee approved all animal handling procedures (case number 08/2020), and the experiment was conducted according to the experimental protocol for the use of live birds from the Brazilian College of Animal Experimentation.

2.2. Birds, experimental design, and diets

The experiment was conducted in Viçosa, MG, Brazil (20°45'57.19" S, 42°51'35.42" W, and 682 m altitude). Male broiler chickens (Cobb 500) used in the experiment were obtained from a commercial hatchery (Rivelli Alimentos SA, Matheus Leme, MG, Brazil), and they were vaccinated against bursal disease and Marek's disease (Serotype 3, Live Marek's Disease Vector, Merial Inc., Athens, GA). From one day of age until the beginning of the experiment, the birds were reared on floor pens (200 × 100 cm) equipped with two nipple drinkers and a feed dispenser. They had free access to water and were fed *ad libitum* with a corn/soybean meal-based diet in mashed form that was formulated to meet the nutritional recommendations of Rostagno et al. (2017).

At 18 days of age, a total of 120 male broilers were assigned based on their body weight to a completely randomized experimental design with three treatments, eight repetitions, and five birds per experimental unit. The birds were housed in 24 experimental units consisting of wire cages (600 cm^2/bird) in a four-level battery equipped with a trough feeder and a nipple drinker.

The treatments were a basal diet or a basal diet with the addition of 1 or 2 mg of capsaicinoids/kg of diet. The corn/soybean meal basal diet was formulated to meet the nutritional recommendations of Rostagno et al. (2017; Table 1). The source of capsaicinoids used was Capcin® (ID4Feed, France), which had a concentration of 5 g capsaicinoids/kg; this product was used at a concentration of 200 and 400 mg/kg of basal diet. The diets were prepared in mashed form. The birds had free access to water and feed throughout the experimental period (18 to 26 days of age).

The ambient temperature was maintained at 22 °C and the birds were exposed to 18 h of continuous light daily during the experimental period.

Table 1 - Ingredients and nutrient composition of the basal diet (as fed basis)

Ingredient (g/kg)	18-26 days of age
Corn	609.8
Soybean meal	311.8
Soybean oil	41.1
Dicalcium phosphate	15.0
Limestone	7.2
Salt	4.9
DL-Methionine, 999 g/kg	3.1
L-Lysine HCl, 780 g/kg	2.8
Vitamin premix[1]	1.2
Trace mineral premix[2]	1.0
L-Threonine, 985 g/kg	0.9
L-Valine, 990 g/kg	0.7
Choline chloride, 600 g/kg	0.5
Calculated composition (g/kg, unless shown)	
Metabolizable energy (MJ/kg)	13.18
Crude protein	195.0
Calcium	7.5
Available phosphorous	3.7
Sodium	2.1
Digestible lysine	11.2
Digestible methionine + cysteine	8.3
Digestible valine	8.7
Digestible threonine	7.4

[1] Vitamin premix provided per kg of diet: vitamin A, 11,564 IU; vitamin D3, 2891 IU; vitamin E, 43.3 IU; vitamin K3, 2.32 mg; vitamin B1, 3.11 mg; vitamin B12, 0.019 mg; vitamin B6, 4.33 mg; vitamin B5, 15.5 mg; vitamin B3, 47.0 mg; vitamin B9, 1.08 mg; biotin, 0.11 mg.
[2] Trace mineral premix provided per kg of diet: Mn, 58.36 mg; Zn, 54.21 mg; Fe, 41.68 mg; Cu, 8.31 mg; I, 0.843 mg; Se, 0.250 mg.

2.3. Performance and sample collection

Broiler performance was evaluated at 26 days of age by determining body weight gain (BWG), feed intake (FI), and feed conversion rate (FCR).

At 26 days of age, one male bird with a weight closest to the average weight of the experimental unit was selected for sample collection. Blood was collected from this bird through the wing vein. The blood was centrifuged at 3,600 × *g* at 4 °C for 10 min for separation; serum samples were stored at −20 °C until analysis. After blood collection, the bird was euthanized by cervical displacement. Samples of the jejunum of 2 cm in length were collected, stored individually in cryogenic tubes, and then placed in liquid nitrogen. Subsequently, they were transferred to freezer storage at −80 °C until the RNA extraction process.

2.4. Serum parameter measurements

The collected blood was used to analyze serum levels of glucose, triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL; Cobas c 311; Roche Diagnostics GmbH, Basel, Switzerland) following the manufacturer's instructions. To measure the serum level of malondialdehyde (MDA), 2.5 mL of 20% trichloroacetic

acid and 1.0 mL of 0.67% thiobarbituric acid were added to 0.5 mL of serum, and the mixture was heated for 30 min in boiling water. The resulting chromogen was extracted with 4.0 mL of n-butyl alcohol. The absorbance of the organic phase was determined at a wavelength of 530 nm.

2.5. Determination of mRNA content

Total RNA was extracted from 50 mg of jejunum samples using TRIzol® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The resulting precipitate was rehydrated with 25 µL of UltraPure DNase/RNase-Free water. RNA concentration was estimated using a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, Beverly, MA, USA), and RNA integrity was determined in a 1.0% agarose gel. The first cDNA strand was synthesized using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Thermo Fisher Scientific, Beverly, MA, USA). The primer sets used are shown in Table 2. β-actin was used as the reference gene for data normalization because of its higher expression and stability. The following target genes were assessed: nuclear factor-κB (NF-κB), glutathione peroxidase (GPX), and Cu, Zn-superoxide dismutase (SOD1). The RT-qPCR analyses were performed in duplicate using Applied Biosystems™ QuantStudio Real-Time PCR Systems (Applied Biosystems, Thermo Fisher Scientific, Beverly, MA, USA) with the relative quantification method, and applying the SYBR® Green system (Applied Biosystems - Foster City, CA, USA) and the GoTaq® qPCR Master Mix kit (Promega Corporation, Madison, USA). Polymerase chain reactions were conducted using the following the program: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The threshold cycle (Ct) values obtained were later normalized (ΔCt) based on the Ct values of the endogenous control gene β-ACT. The calculation of the relative gene expression levels was performed according to the $2^{-\Delta Ct}$ method described by Livak and Schmittgen (2001).

Table 2 - Primer sequences

Gene	Forward sequence	Reverse sequence
NF-κB	GTGTGAAGAAACGGGAACTG	GGCACGGTTGTCATAGATGG
GPX	GACCAACCCGCAGTACATCA	GAGGTGCGGGCTTTCCTTTA
SOD1	AGGGGGTCATCCACTTCC	CCCATTTGTGTTGTCTCCAA
β-actin	TGCTGTGTTCCCATCTATCG	TTGGTGACAATACCGTGTTCA

NF-κB - nuclear factor-κB; *GPX* - glutathione peroxidase; *SOD1* - Cu, Zn-superoxide dismutase.

2.6. Statistical analysis

For each variable, the analysis of variance was performed according to the following general model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

in which Y_{ij} is the measured dependent variable, µ is the overall mean, α_i is the effect of treatments, and ε_{ij} is the random error.

The average FI and BWG of the five birds in each cage were considered for the statistical analysis of the growth performance variables. For serum and mRNA expression analyses, one bird per replicate was considered the experimental unit. Analysis of variance for each variable was performed under a completely randomized design using the GLM procedure of SAS (Statistical Analysis System, 9.4). The analysis of variance assumptions given by normality of residuals and homogeneity of variances were evaluated using the Shapiro-Wilk and Hartley tests, respectively. For the mRNA expression of GPX and SOD1, since the analysis of variance assumptions were not verified, the original data were transformed by Box-Cox transformation. The comparisons

between the means of treatments were performed through Tukey's test, considering a 5% probability of type I error.

3. Results

3.1. Performance

There was no effect of capsaicinoid supplementation on the performance of broilers from 18 to 26 days of age (P>0.05; Table 3).

Table 3 - Growth performance of broilers from 18 to 26 days of age

	Capsaicinoids (mg/kg of diet)			SEM	P-value
	0	1	2		
IBW (kg)	0.652	0.651	0.651	0.002	0.944
BWG (kg/bird/day)	0.076	0.078	0.077	0.001	0.338
FI (kg/bird/day)	0.119	0.122	0.116	0.001	0.392
FCR	1.56	1.56	1.51	0.03	0.691

IBW - initial body weight; BWG - body weight gain; FI - feed intake; FCR - feed conversion ratio; SEM - standard error of the means (n = 8 for treatment).

3.2. Serum metabolites

There was no effect of treatment on serum levels of glucose, triglycerides, cholesterol, HDL, LDL, VLDL, or MDA (P>0.05; Table 4).

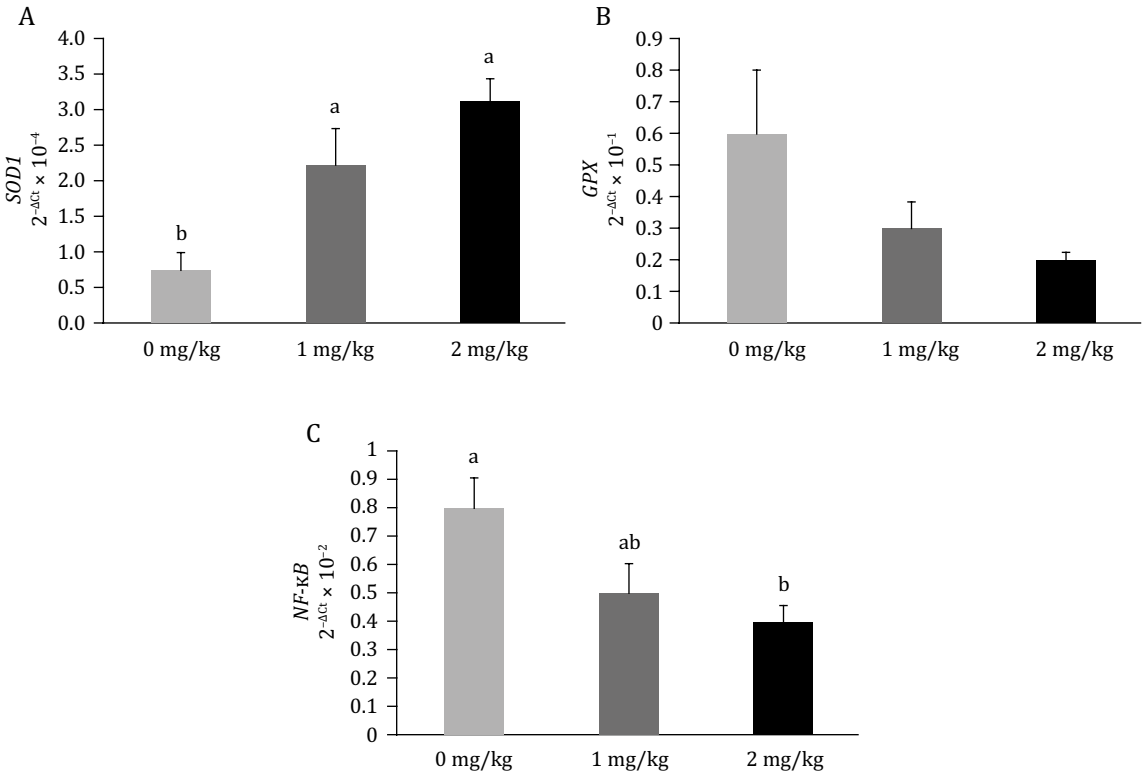
Table 4 - Serum metabolites of broilers at 26 days of age

	Capsaicinoids (mg/kg of diet)			SEM	P-value
	0	1	2		
Glucose (mg/dL)	246.0	242.3	250.6	2.7	0.473
Triglycerides (mg/dL)	87.8	99.3	101.1	5.6	0.598
Cholesterol (mg/dL)	119.8	119.5	119.1	2.4	0.995
HDL (mg/dL)	79.8	79.9	77.6	1.6	0.822
LDL (mg/dL)	22.5	19.8	21.3	1.3	0.710
VLDL (mg/dL)	17.6	19.9	20.2	1.1	0.598
Malondialdehyde (nmol/mL)	3.15	2.95	2.90	0.13	0.740

HDL - high-density lipoprotein; LDL - low-density lipoprotein; VLDL - very low-density lipoprotein; SEM - standard error of the means (n = 8 for treatment).

3.3. mRNA content

Broilers supplemented with 1 and 2 mg/kg capsaicinoids showed higher mRNA expression of SOD1 (P<0.001) in the jejunum compared with birds fed the basal diet (Figure 1). However, the mRNA expression of GPX (P>0.05) was not influenced by the treatments. The capsaicinoid supplementation of 2 mg/kg reduced the mRNA expression of NF-κB (P = 0.031) in the jejunum of broilers compared with the basal diet.



Means (+ standard error of mean) are shown. n = 8 per treatment.
SOD1, P<0.001; *GPX*, P = 0.193; *NF-κB*, P = 0.031.

Figure 1 - The mRNA expression of Cu,Zn-superoxide dismutase (*SOD1*), glutathione peroxidase (*GPX*), and nuclear factor-κB (*NF-κB*) in the jejunum of broilers at 26 days of age in response to the addition of 0, 1 or 2 mg capsaicinoids/kg of diet.

4. Discussion

In this study, we hypothesized that capsaicinoids have antioxidant effects in broilers. In fact, supplementation with 1 or 2 mg capsaicinoids/kg of diet improved the expression of SOD1 in the jejunum. Superoxide dismutase is part of the antioxidant enzyme system, and the increase in its activity in response to exogenous antioxidants has been associated with the removal of ROS from cells (Winiarska-Mieczan et al., 2021). Another important finding in the present study was the reduction in the mRNA expression of NF-κB in the jejunum of broilers supplemented with 2 mg capsaicinoids/kg of diet. The regulatory roles of NF-κB in poultry are still poorly understood, but the available information indicates that, similar to its role in mammals, it is a main regulator of many processes, including inflammation (Surai et al., 2021). Several stress signals have been associated with increased NF-κB, including ROS (Sivandzade et al., 2019). Recently, Liu et al. (2021) also observed an influence of capsaicinoids on the antioxidant status of broilers. The authors observed that birds supplemented with 80 mg/kg natural capsaicin extract had higher levels of serum total antioxidant capacity and SOD and lower levels of the proinflammatory cytokines interleukin-1β and tumor necrosis factor-α.

Malondialdehyde is a marker of lipid peroxidation, and higher levels are due to oxidative stress in the body (Ghasemi-Sadabadi et al., 2020). However, despite the above results, supplementation with capsaicinoids did not influence the serum concentration of MDA or other evaluated metabolites. In contrast to the results observed in the present study, supplementation with hot red pepper oil decreased the plasma levels of triglycerides, cholesterol, and LDL (Liu et al., 2021; Hassan et al., 2022).

In the present study, capsaicinoid supplementation did not improve the performance of broilers, and this finding was different from previous reports. Hassan and El-Ktany (2020) observed that the supplementation of 0.25 to 1 mL/kg of hot red pepper oil improved the weight gain and FCR of broilers. Thiamhirunsopit et al. (2014) also observed improvements in the weight gain and FCR of broilers fed a diet containing 20 mg/kg capsaicin. The positive effect on performance may be associated with the antioxidant capacities and anti-inflammatory activities of capsaicinoids (Nascimento et al., 2013). In addition, Liu et al. (2021) associated the better performance with enhanced growth hormone levels observed in broilers fed diets containing natural capsaicin extract. The lack of positive results on the performance in the present study may be due to the short experimental period that limited the response time of the birds.

5. Conclusions

Supplementation with 1 and 2 mg/kg capsaicinoids did not improve the performance of broilers from 18 to 26 days of age but increased the mRNA expression of SOD1 and reduced the mRNA expression of NF-κB in the jejunum of broilers.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: B.S. Kreuz, M.S. Duarte and A.A. Calderano. Data curation: S.O. Borges and A.A. Calderano. Formal analysis: B.S. Kreuz, M.S. Duarte and A.A. Calderano. Funding acquisition: A.A. Calderano. Investigation: B.S. Kreuz, S.O. Borges, M.C.N. Piazza, M.E.S. Carvalho, J.V.S. Miranda and A.A. Calderano. Methodology: B.S. Kreuz and A.A. Calderano. Project administration: L.F.T. Albino and A.A. Calderano. Supervision: M.S. Duarte, L.F.T. Albino and A.A. Calderano. Validation: A.A. Calderano. Visualization: A.A. Calderano. Writing – original draft: B.S. Kreuz. Writing – review & editing: M.S. Duarte, L.F.T. Albino and A.A. Calderano.

Acknowledgments

This work has been supported by the following Brazilian research agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Bacou, E.; Walk, C.; Rider, S.; Litta, G. and Perez-Calvo, E. 2021. Dietary oxidative distress: a review of nutritional challenges as models for poultry, swine and fish. Antioxidants 10:525. https://doi.org/10.3390/antiox10040525

Chalvon-Demersay, T.; Luise, D.; Le Floc'h, N.; Tesseraud, S.; Lambert, W.; Bosi, P.; Trevisi, P.; Beaumont, M. and Corrent, E. 2021. Functional amino acids in pigs and chickens: implication for gut health. Frontiers in Veterinary Science 8:663727. https://doi.org/10.3389/fvets.2021.663727

Ghasemi-Sadabadi, M.; Veldkamp, T.; van Krimpen, M.; Ebrahimnezhad, Y.; Ghalehkandi, J. G.; Salehi, A.; Didehvar, M.; Khodaei, M. and Mehdizadeh, A. 2020. Determining tolerance of Japanese quail to different dietary fat peroxidation values by supplementation with Rosemary and Aloe Vera on performance and meat quality. Animal Feed Science and Technology 267:114574. https://doi.org/10.1016/j.anifeedsci.2020.114574

Giuffrida, D.; Dugo, P.; Torre, G.; Bignardi, C.; Cavazza, A.; Corradini, C. and Dugo, G. 2013. Characterization of 12 *Capsicum* varieties by evaluation of their carotenoid profile and pungency determination. Food Chemistry 140:794-802. https://doi.org/10.1016/j.foodchem.2012.09.060

Hassan, S. S. A. and El-Ktany, E. M. 2020. Effect of hot red pepper oil on the productivity, carcass characteristics and economic efficiency of broiler chickens. Journal of Animal Health and Production 9(s1):128-134. https://doi.org/10.17582/journal.jahp/2020/9.s1.128.134

Hassan, S.; Hassan, M.; Soliman, F. and Safwat, A. 2022. Influence of hot red pepper oil in broiler diets on blood, antioxidant, immunological parameters and intestinal bacteria counts. Animal Biotechnology. https://doi.org/10.1080/10495398.2021.2020132

Hernández-Pérez, T.; Gómez-García, M. R.; Valverde, M. E. and Paredes-López, O. 2020. *Capsicum annuum* (hot pepper): an ancient Latin-American crop with outstanding bioactive compounds and nutraceutical potential. A review. Comprehensive Reviews in Food Science and Food Safety 19:2972-2993. https://doi.org/10.1111/1541-4337.12634

Karadas, F.; Pirgozliev, V.; Rose, S. P.; Dimitrov, D.; Oduguwa, O. and Bravo, D. 2014. Dietary essential oils improve the hepatic antioxidative status of broiler chickens. British Poultry Science 55:329-334. https://doi.org/10.1080/00071668.2014.891098

Liu, S. J.; Wang, J.; He, T. F.; Liu, H. S. and Piao, X. S. 2021. Effects of natural capsicum extract on growth performance, nutrient utilization, antioxidant status, immune function, and meat quality in broilers. Poultry Science 100:101301. https://doi.org/10.1016/j.psj.2021.101301

Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402-408. https://doi.org/10.1006/meth.2001.1262

Mishra, B. and Jha, R. 2019. Oxidative stress in the poultry gut: potential challenges and interventions. Frontiers in Veterinary Science 6:60. https://doi.org/10.3389/fvets.2019.00060

Nascimento, P. L. A.; Nascimento, T. C. E. S.; Ramos, N. S. M.; Silva, G. R.; Camara, C. A.; Silva, T. M. S.; Moreira, K. A. and Porto, A. L. F. 2013. Antimicrobial and antioxidant activities of Pimenta malagueta (*Capsicum frutescens*). African Journal of Microbiology Research 7:3526-3533.

Patra, A. K. 2020. Influence of plant bioactive compounds on intestinal epithelial barrier in poultry. Mini-Reviews in Medicinal Chemistry 20:566-577. https://doi.org/10.2174/1389557520066191226111405

Pirgozliev, V.; Mansbridge, S. C.; Rose, S. P.; Lillehoj, H. S. and Bravo, D. 2019. Immune modulation, growth performance, and nutrient retention in broiler chickens fed a blend of phytogenic feed additives. Poultry Science 98:3443-3449. https://doi.org/10.3382/ps/pey472

Rostagno, H. S.; Albino, L. F. T.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saraiva, A.; Teixeira, M. L.; Rodrigues, P. B.; Oliveira, R. F.; Barreto, S. L. T. and Brito, C. O. 2017. Tabelas Brasileiras para suínos e aves: Composição de alimentos e exigências nutricionais. 4.ed. Departamento de Zootecnia, UFV, Viçosa, MG.

Sivandzade, F.; Prasad, S.; Bhalerao, A. and Cucullo, L. 2019. NRF2 and NF-κB interplay in cerebrovascular and neurodegenerative disorders: molecular mechanisms and possible therapeutic approaches. Redox Biology 21:101059. https://doi.org/10.1016/j.redox.2018.11.017

Surai, P. F.; Kochish, I. I. and Kidd, M. T. 2021. Redox homeostasis in poultry: regulatory roles of NF-κB. Antioxidants 10:186. https://doi.org/10.3390/antiox10020186

Thiamhirunsopit, K.; Phisalaphong, C.; Boonkird, S. and Kijparkorn, S. 2014. Effect of chili meal (*Capsicum frutescens* LINN.) on growth performance, stress index, lipid peroxidation and ileal nutrient digestibility in broilers reared under high stocking density condition. Animal Feed Science and Technology 192:90-100. https://doi.org/10.1016/j.anifeedsci.2014.03.009

Winiarska-Mieczan, A.; Kwiecień, M.; Mieczan, T.; Kwiatkowska, K. and Jachimowicz, K. 2021. The effect of Cu, Zn and Fe chelates on the antioxidative status of thigh meat of broiler chickens. Animal 15:100367. https://doi.org/10.1016/j.animal.2021.100367

Xue, F.; Shi, L.; Li, Y.; Ni, A.; Ma, H.; Sun, Y. and Chen, J. 2020. Effects of replacing dietary Aureomycin with a combination of plant essential oils on production performance and gastrointestinal health of broilers. Poultry Science 99:4521-4529. https://doi.org/10.1016/j.psj.2020.05.030